signed to study groups but did not undergo PTCA, and there were 20 procedural failures in each group. An additional 16 patients experienced a complication while in the hospital. Eight of these were ischemic events requiring emergency revascularization within 48 hours of the primary PTCA — four by a repeated PTCA and four by coronary-bypass surgery; these events were evenly distributed according to drug group. The remaining eight experienced a variety of early intercurrent events necessitating discontinuation of the study medication. In three patients the medication was stopped because of upper gastrointestinal symptoms, in two for hematologic reasons, in two because of chest pain (one in each treatment group), and in one because of nonadherence to the study protocol by the angioplasty operator. These patients, although ineligible for restenosis evaluation, were nevertheless assessed for periprocedural events.

Forty-two patients did not undergo final quantitative coronary angiography during the period between four and seven months after PTCA. Twenty-seven of these patients or their private physicians refused the procedure, 3 underwent elective coronary-bypass surgery, and 12 had angiography before four months had elapsed. Ten of the 12 chose not to return for a later study. The other two patients (both taking placebo) had obstructions of 90 and 100 percent, respectively,

Table 2. Demographic, Clinical, and Angiographic Characteristics of the Two Study Groups.*

Characteristic†	Aspirin–Dipyridamole Group	Placebo Group	P Value‡
Patients randomized	187	189	
Age (yr)	52.1±9.2	52.0±8.9	0.91
Female (%)	24.6	16.4	0.05
Current smoker (%)	27.8	29.6	0.70
History of hypertension (%)	20.9	18.1	0.50
LDL cholesterol (mmol/liter)	3.7±1.1	3.8±1.1	0.35
Diabetes (%)	4.3	8.5	0.10
Angina class			
I	10.2	6.4	0.57
II	42.4	41.3	
III	40.7	44.2	
IV	6.8	8.1	
Unstable angina (%)	15.5	14.3	0.74
Previous myocardial infarction (%)	25.1	24.3	0.87
Extent of coronary artery disease (%)§			
1 Vessel	66	65	0.34
2 Vessels	30	27	
3 Vessels	4	8	
Segments in which PTCA was attempted	235	258	
LAD/RCA/CCA (% distribution)	53/32/15	49/31/20	0.31
Degree of stenosis (%)			
<70	13	13	0.89
70–89	51	49	
90–99	33	36	
100	3	2	
Patients in whom PTCA was attempted	185	184	
Single-lesion angioplasty (%)	76	72	0.39
Multiple-lesion angioplasty (%)	24	28	

*Plus–minus values are means ±SD.

†LDL denotes serum low-density lipoproteins, LAD left anterior descending artery, RCA right coronary artery, and CCA circumflex coronary artery. Multiple-lesion angioplasty refers to either single-vessel multiple-lesion angioplasty or multiple-vessel angioplasty.

‡P values refer to the difference in prevalence between the two groups.

§Coronary artery disease was defined as stenosis of 50 percent or more by visual estimation.

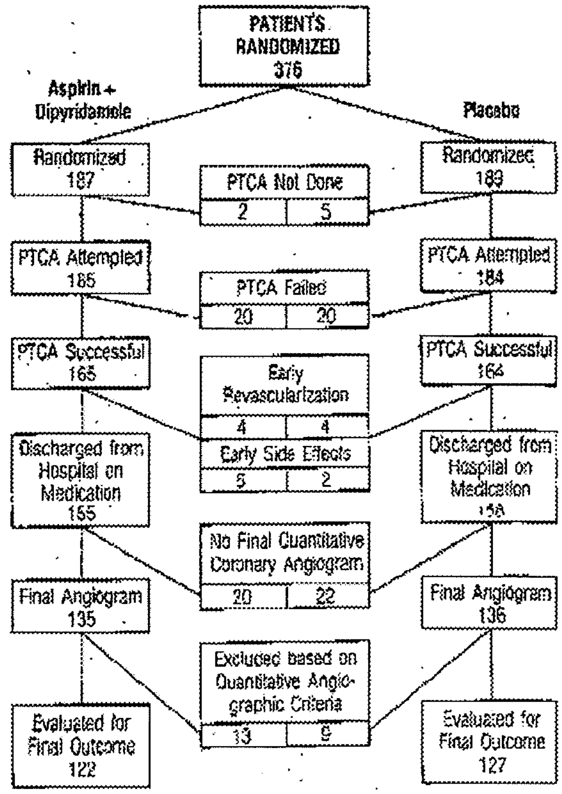

Figure 1. Patient Flow, with Reasons for Excluding Patients before Final Evaluation.

"Excluded based on quantitative angiographic criteria" refers to patients who lacked at least one lesion with a diameter of stenosis of 50 percent or more that was reduced to less than 50 percent after PTCA, as measured by quantitative angiography.

by visual estimation at the time of their follow-up angiography, but for administrative reasons, no quantitative assessment of the angiograms was available.

Twenty-two patients were excluded because they did not fulfill the quantitative criteria for a successful PTCA, even though at the time of their PTCA it was considered successful by visual estimation and they were retained in the study.

The demographic, clinical, and angiographic characteristics examined in the 376 randomized patients were also evaluated in the 249 patients who completed the study. There were no statistically significant differences between the two groups.

Rate of Restenosis

The rate of restenosis was analyzed in the 249 patients (284 segments) who underwent final angiography. The restenosis rates were almost identical in the two treatment groups, whether expressed in terms of segment or of patient (Fig. 2). The use of a more stringent definition of segment restenosis (requiring in addition at least a 10 percent increase in the diameter of stenosis between the measurement made immedi-

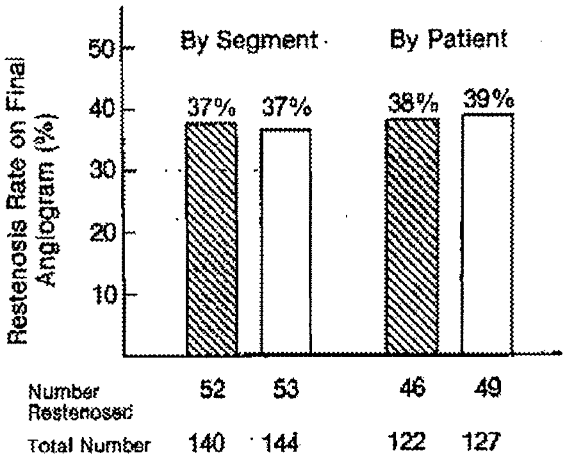

Figure 2. Rate of Restenosis, According to Segment and According to Patient, in the Aspirin–Dipyridamole Group (Hatched Bars) and the Placebo Group (Open Bars).

ately after PTCA and that made at final angiography) did not alter the results. Furthermore, when other definitions of restenosis were used, such as a loss of at least 50 percent of the gain in luminal diameter achieved by PTCA, the findings were very similar. In addition, the mean minimum absolute diameter of stenosis before PTCA, after PTCA, and at final angiography did not differ in the patients taking the active drug and those taking placebo (Fig. 3). There were no differences in the incidence of recurrent angina or positive exercise stress tests in the two treatment groups during follow-up.

Periprocedural Events

A periprocedural event occurred in 27 of the 376 randomized patients (7.2 percent) (Table 3). This complication rate was similar to that reported recently from the PTCA registry of the National Heart, Lung, and Blood Institute.[19] The incidence of Q-wave myocardial infarction was 6.9 percent (13 patients) in the placebo group and 1.6 percent (3 patients) in the drug group — a statistically significant difference ($P = 0.0113$). The rates of early revascularization, nine events in each group, were virtually identical (Table 3). All but one of these early events were characterized by chest pain and rapidly evolving electrocardiographic changes. In 20 of the 27 patients, ischemia appeared within 6 hours of PTCA, and in the others, between 6 and 14 hours. Bypass surgery was performed an average of 4 hours after PTCA, with a range of 2 to 14 hours, and PTCA was repeated an average of 8 hours after the index PTCA, with a range of 1 to 12 hours.

Safety Monitoring

The Operations Committee of the study, after periodic review of the electrocardiographic and enzyme data, concluded in January 1987 that a difference had emerged in favor of the active drug with regard to the incidence of myocardial infarction, and requested that enrollment be terminated for ethical reasons. Thus, 284 segments underwent final evaluation, with a restenosis rate of 37 percent in the placebo group. When a two-sided test was used at the 0.05 level of significance, the retrospective power to detect a 50 percent reduction in restenosis was 0.86, with a corresponding beta error of 0.14.

Side Effects and Compliance with Study Medication

As shown in Table 4, gastrointestinal side effects were slightly more common in patients on the active drug, whereas the rate of other side effects was similar in both treatment groups. Of 155 patients taking the active drug when they left the hospital, 18 (11.6 percent) stopped taking their medication because of side effects, whereas this occurred in 14 of 158 patients taking placebo (8.9 percent). This difference is not statistically significant ($P = 0.43$).

Compliance as assessed by pill count is shown in Table 5 and was good throughout the trial.

Discussion

To date, all controlled studies investigating the efficacy of pharmacologic agents in reducing restenosis after coronary angioplasty have had negative results.[20-22] This study was a placebo-controlled trial designed to evaluate the role of antiplatelet agents in patients undergoing PTCA.

Several practical problems were encountered during the trial. First, the recruitment of patients was somewhat delayed because of the recent rapid increase in the use of antiplatelet and anticoagulant agents among patients with coronary disease, particularly those with unstable angina. Second, the study design required the initiation of treatment before PTCA, since pretreatment might be necessary

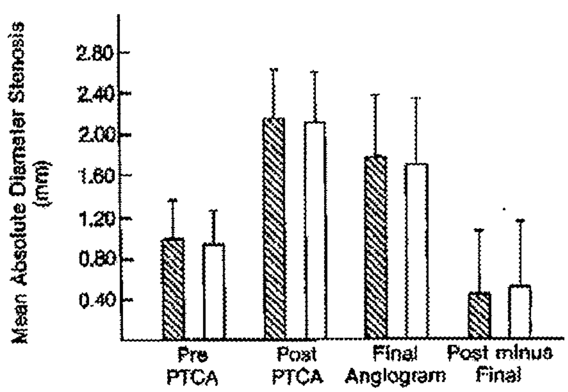

Figure 3. Mean Absolute Diameter of Stenosis, as Measured Throughout the Study for the 284 Segments Undergoing Final Angiography in the Aspirin–Dipyridamole Group (Hatched Bars) and the Placebo Group (Open Bars).

Bars represent means ±SD. No significant differences were found between treatment groups.

Table 3. Major Periprocedural Events.

Event	Aspirin–Dipyridamole Group (N = 187)	Placebo Group (N = 189)
Q-wave myocardial infarction	1	8
Q-wave myocardial infarction with early revascularization*	2	5
Total†	3	13
Early revascularization without Q-wave myocardial infarction	7	4

*Early revascularization denotes coronary-artery bypass surgery or PTCA repeated within 48 hours of the index PTCA.

†P = 0.0113 by Pearson chi-square test.

for efficacy.[23,24] The practical implication of this design is an unavoidable dropout rate of 10 to 20 percent at the outset, however, because of procedural failure and complications necessitating surgery. Third, side effects of the study medication and restrictions imposed by the quantitative coronary angiographic criteria resulted in further exclusions. Nonetheless, quantitative angiography was considered essential for accurate evaluation of restenosis. The result of this attrition was that although the effect of the active drug on periprocedural events could be evaluated on an intention-to-treat basis in the 376 randomized patients, its effect on restenosis could be analyzed only in the 249 patients who had successful angioplasties and reached the stage of final quantitative angiography.

The ancillary medication used during the angioplasty procedure and the method used to administer the study medication require comment. Dextran is rarely used during angioplasty today because of its probable inefficacy,[25] but it was part of the standard procedural protocol at the beginning of our trial. Similarly, the heparin regimen followed here was in use at the two participating institutions and at several others in 1983. The relatively low dose of heparin after the procedure was chosen to minimize the risk of groin hematoma. A higher dose might have reduced the incidence of early complications, but there are no studies to substantiate this. Finally, regarding the study medication, a combination capsule was used to improve compliance, and intravenous rather than oral

Table 4. Incidence of Side Effects of Study Medication among Patients Taking Medication after Discharge from the Hospital.

Side Effect	Aspirin–Dipyridamole Group (N = 155)	Placebo Group (N = 158)	P Value*
	number (percent)		
Gastrointestinal upset	54 (34.8)	36 (22.8)	0.02
Headache	10 (6.5)	10 (6.3)	0.96
Bleeding	2 (1.3)	0 (0.0)	0.15
Other	20 (12.9)	17 (10.8)	0.56

*P values were derived with use of the Pearson chi-square test.

dipyridamole was administered on the day of the PTCA to ensure acceptable blood levels for the procedure, taking into account the variable gastrointestinal absorption of this drug.

The significantly lower incidence of in-hospital Q-wave myocardial infarction in our patients assigned to antiplatelet drugs accords with previous experimental data. In experiments with animals, marked platelet accumulation occurs at peripheral sites of angioplasty after 30 minutes, persists for up to four hours, and is most excessive when there is increased angiographic evidence of dissection.[14] Furthermore, antiplatelet therapy given before PTCA — either low-dose aspirin or aspirin with dipyridamole — has been associated with decreased deposition of platelets and a lower prevalence of mural thrombus formation.[26] Our findings are also consistent with those of a recently reported retrospective clinical study comparing the effect of various antiplatelet regimens on angiographic abnormalities and complications occurring during PTCA.[27] In that study, of 28 patients who received both oral aspirin and dipyridamole before and during PTCA, none had angiographic evidence of thrombus

Table 5. Compliance with Study Medication, According to Pill Count.*

No. of Months after PTCA	Aspirin–Dipyridamole Group		Placebo Group	
	No. of patients with counts	% of capsules taken	No. of patients with counts	% of capsules taken
1	103	95±9	106	97±6
3	92	95±6	98	94±9
6	80	93±17	77	93±10

*Plus–minus values are means ±SD.

or required emergency surgery, as opposed to a 21.5 percent incidence of thrombus and a 9.9 percent incidence of emergency surgery in 118 patients who received either no antiplatelet pretreatment or only dipyridamole. Undoubtedly, acute thrombosis is one of the mechanisms of infarction during PTCA and one of the circumstances leading to emergency surgery. These considerations may explain our finding of a lower rate of procedural events in patients taking the active drug. From the design of our study, it is not possible to determine whether smaller doses or a different method of administration of either drug would be equally or more effective — or whether, in fact, both agents are necessary. Recently, doubts have been raised concerning the contribution of dipyridamole to the antithrombotic action of aspirin.[28]

Despite evidence of early benefit, our data failed to indicate that any long-term protection from restenosis was afforded by the combination antiplatelet regimen used in our study. There are at least four possible theoretical explanations for this negative result. First, experimental data have shown that the combination

of aspirin and dipyridamole does not completely prevent platelet adhesion at the site of angioplasty,[26] and this may be an important factor in initiating smooth-muscle proliferation and migration. Second, it is possible that the antiplatelet regimen we used did not reduce platelet accumulation (aggregation) sufficiently. Third, it is conceivable that there was some pharmacologic interference between the two drugs and that either agent alone might have been successful in preventing restenosis. In a randomized study,[20] however, the long-term outcome of patients taking aspirin alone was not substantially better. Finally, it is possible that platelets are not an important factor in the pathogenesis of restenosis in humans.

In conclusion, this prospective randomized, double-blind trial suggests that patients undergoing coronary angioplasty should follow an antiplatelet regimen from 24 hours before until at least 48 hours after the procedure to reduce the incidence of periprocedural myocardial infarction. However, we could not demonstrate any long-term benefit in preventing restenosis of continuing the antiplatelet medication beyond the period of hospitalization.

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Preclinical restenosis models and drug-eluting stents: Still important, still much to learn

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Clinical Trials: Viewpoint

Preclinical Restenosis Models and Drug-Eluting Stents

Still Important, Still Much to Learn

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Percutaneous coronary intervention continues to revolutionize the treatment of coronary atherosclerosis. Restenosis remains a significant problem but may at last be yielding to technologic advances. The examination of neointimal hyperplasia in injured animal artery models has helped in our understanding of angioplasty and stenting mechanisms, and as drug-eluting stent (DES) technologies have arrived, they too have been advanced through the study of animal models. These models are useful for predicting adverse clinical outcomes in patients with DESs because suboptimal animal model studies typically lead to problematic human trials. Similarly, stent thrombosis in animal models suggests stent thrombogenicity in human patients. Equivocal animal model results at six or nine months occasionally have been mirrored by excellent clinical outcomes in patients. The causes of such disparities are unclear but may result from differing methods, including less injury severity than originally described in the models. Ongoing research into animal models will reconcile apparent differences with clinical trials and advance our understanding of how to apply animal models to clinical stenting in the era of DESs. (J Am Coll Cardiol 2004;44:1373–85) © 2004 by the American College of Cardiology Foundation

Percutaneous coronary intervention continues to revolutionize atherosclerosis treatments. The understanding of angioplasty mechanisms came after these technologies were already in clinical use through the comparison animal model research with clinical pathologic specimens. An early understanding of balloon angioplasty suggested that atherosclerotic plaque was "compressed" or "stretched"—concepts that eventually yielded to a comprehensive understanding that both plaque and normal artery are severely fractured in many successful cases (good clinical percutaneous transluminal coronary angioplasty or stent result). Animal models assumed a central position in understanding coronary artery injury and healing. Neointimal formation results from vessel laceration, which is a response to injury during revascularization. Rare but valuable human necropsy material has confirmed animal model results showing that plaque that was fractured or lacerated by coronary angioplasty induced severe arterial injury and that restenosis resulted from this injury (1,2).

Much of what is known about restenosis and neointimal formation comes from intense study of animal injury models and comparison with human material, which usually is derived from autopsy. What is referred to as "restenosis" in normal animal arteries is not truly such; rather, it is neointima resulting from controlled injury that is induced in normal vessels. Stenosis in these models results from thick and sometimes occlusive neointima forming after severe balloon or stent injury and also from vessel shrinkage (remodeling) due to scar formation. As injured normal animal arteries (rat, pig, mouse, dog, rabbit, primate) became the standard for understanding neointima and remodeling, they rapidly evolved into a new role, that of testing novel restenosis therapies (3,4). Many parallels emerged between human restenosis and its animal model counterparts. Each has strongly impacted our understanding of restenosis and its treatment.

From the *Minneapolis Heart Institute and †Minnesota Cardiovascular Research Institute, Minneapolis, Minnesota; ‡American Cardiovascular Research Institute, Atlanta, Georgia; and §Armed Forces Institute of Pathology, Bethesda, Maryland. This manuscript summarizes three lectures presented at a "Meet the Experts" session held at the U.S. Food and Drug Administration entitled "Animal Restenosis Models, What Have We Learned?"

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ANIMAL RESTENOSIS MODELS: A BRIEF OVERVIEW

Rat carotid artery model. The rat carotid artery model was developed in the 1960s, and from it derived the foundations of vascular biology. Although first used to gain insight into human atherosclerosis, it was adapted to understand restenosis and to test restenosis therapy. This model became a standard for studying smooth muscle cell proliferation after endothelial denudation (5–11). One advantage of the model is that it provides one with the ability to study molecular biology (11–14).

This model assumed less importance after several early studies of angiotensin-converting enzyme inhibitors. These agents were very effective at inhibiting neointimal thickening, suggesting the importance of angiotensin II to neointimal growth (15,16). However, two subsequent clinical studies failed to show inhibitory effects (17–19). Angiotensin II has been the subject of ongoing interest (20–22), however, the failure of this model to predict negative clinical trial results has caused it to lose favor among investigators.

Abbreviations and Acronyms

DES	= drug-eluting stent
IVUS	= intravascular ultrasonography
MLD	= minimum lumen diameter
PRESTO	= Prevention of REStenosis with Tranilast and its Outcomes trial

Mouse arterial injury model. The mouse arterial injury as a restenosis model developed from the availability of the mouse genome and molecular methods to study events after arterial injury (23,24). The mouse has very small vessels; therefore, traditional injury methods by balloon or stent are not practical. Injury may instead be performed by rotating a small guidewire in the vessel (25–28) or electrical injury. Either of these methods causes endothelial loss and focal medial cell damage of 25% to 50%. The internal elastic lamina often is disrupted by these injury procedures. Variable neointimal thickening forms focally at injury sites in proportion to the amount of injury, and little thrombus occurs in this model.

Wound healing in the mouse model partially replicates other models because its features include mural thrombus resorption through inflammatory cell infiltration. A thin neointima (roughly 0.03 mm^2) forms by three weeks. Because most or all arterial cells (in media and adventitia) are killed uniformly, these lesions heal from the borders. The power of molecular biology and genetics in these mouse models will permit substantial advances in understanding of the interactions among cell proliferation, cell migration, thrombus formation, and remodeling.

Hypercholesterolemic rabbit iliac model. The rabbit iliac restenosis model also has been studied extensively to test restenosis therapies and to understand cellular and molecular mechanisms (29–31). Blood cholesterol levels are typically >1,000 mg/dl and cause biochemical arterial injury, which is supplemented by mechanical injury.

These models add initial injury by air desiccation to hypercholesterolemic diets and finally balloon inflation to further injure the vessel. Unlike rat carotid arteries, macroscopic and hemodynamically significant stenoses similar to human restenosis develop reliably in the rabbit models. Histopathology in this model shows foam cells (macrophages that have ingested excessive lipid) and voluminous extracellular matrix. One criticism of this model is that foam cells are rare in human restenotic neointima. However, balloon angioplasty in this model does cause histopathologic injury comparable with that of human angioplasty, with medial dissection and plaque fracture.

Platelet deposition occurs rapidly at sites of a balloon-induced plaque fracture. Thus, antiplatelet agents were studied early in the history of this model as a potential therapy (32,33) and showed efficacy in reducing neointimal thickness. A wide variety of other agents have been studied in this model and are discussed later.

Porcine coronary injury model. The coronary arteries of domestic crossbred pigs respond similar to human coronary arteries after injury (34–36). A hypercholesterolemic diet produces lesions more severe in nature than standard laboratory diets (37,38). In this model, injury causes thick neointima within 28 days. The neointima is identical to human restenotic neointima. When a balloon-only injury is performed, a typical medial laceration occurs and is filled at 28 days by neointima. The amount of neointimal thickening is directly proportional to injury. This permits the creation of an injury-response regression relationship that quantitates the response to potential therapies (39–41).

Relevance to human coronary intervention. The porcine coronary models using injuries caused by either stenting or overstretching injury alone are now accepted standards by which potential restenosis therapies are studied, in large part because the stages of neointimal growth described in the porcine model follow those now known in humans. Empiric correlation with clinical trials suggests this may be true. Negative trials using the porcine model correspond well to negative clinical trials, suggesting that this model has good specificity. Fewer therapies have had positive results and, therefore, model sensitivity is less certain. Paclitaxel- and rapamycin-eluting stent studies suggest that positive results in these models are predictive of positive results in clinical trials. Interestingly, ionizing radiation to the coronary arteries in the pig model demonstrated neointimal stimulation rather than inhibition when gamma radiation was delivered externally (42). However, many studies of intravascular gamma and beta radiation show neointimal inhibition in pigs when examined at 28 days after therapy. Longer-term data are less conclusive and suggest little efficacy at longer time points.

Human coronary arteries develop radiation-induced coronary artery disease, although this is achieved typically with high doses of radiation that are given for many years. Several clinical studies in patients receiving vascular brachytherapy for in-stent restenosis show neointimal stimulation at the edges of radiated regions, where radiation doses are falling off. Moreover, several reports are emerging that suggest a "catch-up" phenomenon in patients receiving vascular brachytherapy. Six-month data in pigs showing lack of efficacy might have predicted this clinical finding; further long-term patient analysis is underway to determine potential relationships to the pig model. Continued observation over time will determine whether intravascular brachytherapy will stimulate accelerated coronary artery disease in patients.

Sensitivity for efficacy will be better assessed as additional strategies that are efficacious are developed. The data suggest that the porcine model is best for establishing safety, although efficacy remains less certain as discussed in detail below. Table 1 compares several human trials with preclinical results. This table includes references for brachytherapy (43–62), statins (63–67), angiotensin-converting enzyme inhibitors (18,19,21,68–72), anticoagulants (39,73–87),

Table 1. Comparison Between Clinical Trials and Porcine Preclinical Data

	Porcine Model Safety/Efficacy	Human Data Safety/Efficacy
Brachytherapy	+/+ (43–51)	+/+ (52–62)
Statins	−/− (63)	−/− (64–67)
Angiotensin-converting enzyme inhibitors	−/− (21,69–71)	−/− (18,19,68,72)
Anticoagulants	±/± (39,73–81)	−/− (82–87)
Probucol	+/+ (88–94)	−/− (95–97)
Rapamycin/analogs	+/+ (98–100)	+/+ (101–105)
Paclitaxel	+/+ (106–110)	+/+ (111–115,177)
Calcium channel blockers	±/− (116)	−/− (117–119)
c-myc antisense	+/+ (120,121)	−/− (122,123)
Dexamethasone	+/+ (124,125)	(126–128)
Heparin	(73,80,81,129,130)	(131–133)

Data in parentheses are reference numbers.

probucol (88–97), rapamycin (98–105), paclitaxel (106–115), calcium channel blockade (116–119), antisense (120–123), dexamethasone (124–128), and heparin (74,80,81,129–133).

THE PROPORTIONALITY BETWEEN INJURY AND NEOINTIMAL THICKENING

Fundamentally, mature neointima is a repaired artery and thus is desirable. Problems arise in only a minority of cases when exuberant neointima impinges on luminal blood flow. Early studies in the porcine coronary artery injury model suggested that deeper arterial injury results in greater neointimal thickening (35). This proportionality in the pig model was subsequently sought and validated in patients (Fig. 1). A practical outcome of this phenomenon was improved stent design, which sought to induce less arterial injury (134,135). Early wire stents could cause substantial injury if they were overexpanded; slotted tubular designs created fewer injuries and have prevailed in modern stent designs (36). Other stent concepts have attempted to limit injury even more but have been less successful, likely because a 90% stenosis when properly dilated undergoes 10-fold expansion. This expansion induces significant, unavoidable arterial injury by necessity and occurs both with angioplasty alone and with stenting. Drug-eluting stents (DESs) also induce such injury but rely on local drug effects to moderate the neointimal response.

Overstretch injury to pig coronary arteries holds important lessons for neointimal response to injury. Simple overstretch without stent implant usually causes medial fracture and laceration, with frequent dissections. A typical balloon:artery ratio is 1.2:1 or 1.3:1, which is visually estimated by the operator. These ratios generally create enough injury for satisfactory neointimal thickness without the risk of large dissections. Larger balloon:artery ratios yield a high likelihood of severe dissection with resulting thrombosis, coronary occlusion, and ensuing death from myocardial infarction and ventricular fibrillation. These balloon:artery ratios are finding use in DES efficacy studies.

When stents are implanted, dissections usually are controlled except at the stent margins. However, stent:artery

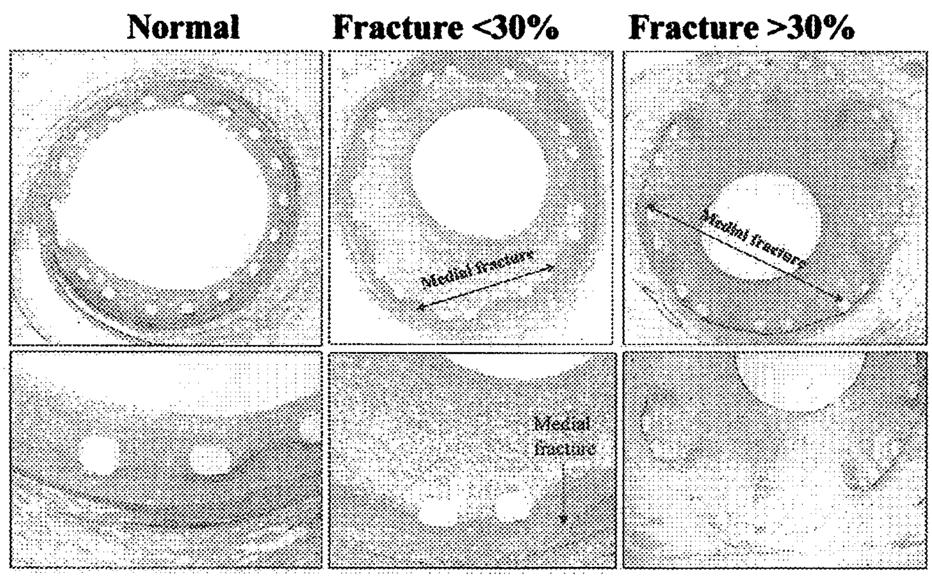

Figure 1. Stent-induced arterial injury in patients generates a proportional neointimal response. **Panels from left to right** indicate that as the internal elastic lamina becomes more severely disrupted by the stent and as the proportion of medial fracture transitions from <30% to >30% **(middle and right columns)**, neointimal growth becomes progressively more severe.

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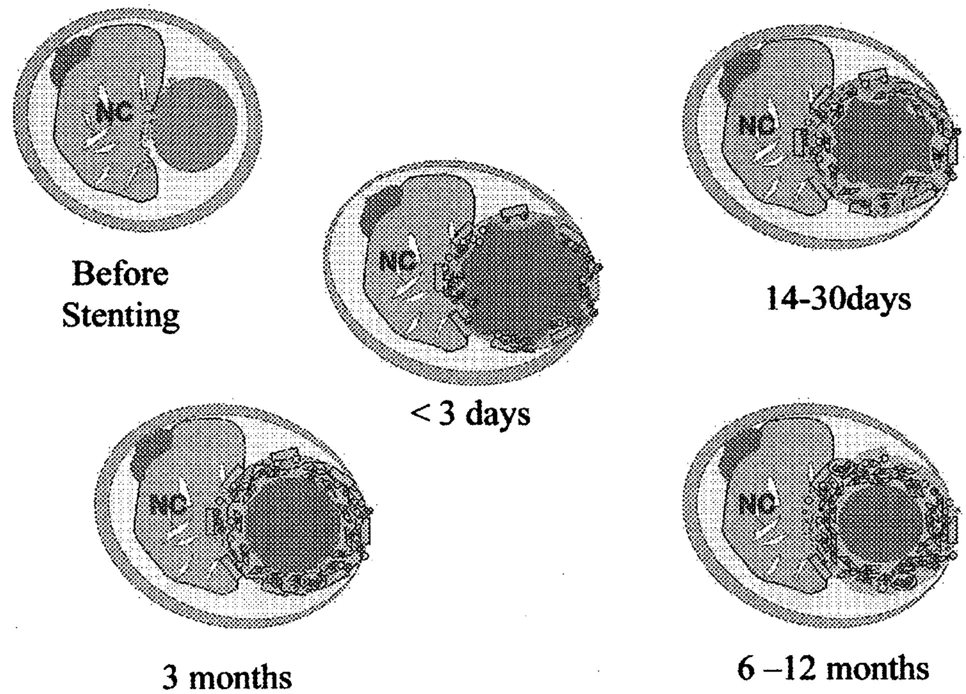

Figure 2. Diagram illustrating the time course of events leading to neointimal hyperplasia in atherosclerotic human coronary arteries. In the first stage, the atherosclerotic artery is depicted before stent placement. NC = non-cellular region of the plaque. Within the first three days after stent placement, platelets/fibrin and neutrophils accumulate at the stent site. At 14 to 30 days, chronic inflammation develops (macrophages, lymphocytes) and persistent fibrin is visible. Smooth muscle cells also are beginning to appear within the stent. At three months, chronic inflammation remains, and fibrin frequently persists. Proteoglycan and matrix deposition occurs. At 6 to 12 months, there often is persistent, chronic inflammation close to the struts, and endothelialization generally is complete. A neointima rich in smooth muscle cells, with a proteoglycan and collagen matrix, has developed. Adapted from Virmani et al. (136).

ratios of >1.3:1 often cause chronic vessel injury as the stent struts migrate through the vessel wall, including through the external elastic lamina and adventitia. Marked inflammation accompanies the stent struts when such oversizing is performed. This inflammation is highly undesirable because drug elution cannot overcome such severe and chronic injury, making stent/drug efficacy assessment not possible. It is for this reason that in preclinical DES testing, a more common practice is to use the balloon:artery ratio of 1.1:1, with the resulting data applied to safety analysis but not efficacy because neointimal generation at these low injury levels is minimal to mild. The relation of safety and efficacy studies with stented and overstretching alone remains to be determined. Figure 2 summarizes the time course of neointimal hyperplasia after stenting in patients. The important steps are summarized in the following text, as learned from animal models and translated to patients (136,137).

Thrombus and restenosis. Mural thrombus in porcine models is an early response to balloon dilation and stenting. It occurs less often in injured rat and dog arteries (138). A direct relationship between thrombus volume and neointimal volume is unproven but is thought likely.

Fibrin- and platelet-rich thrombus form on stent struts in porcine arteries within hours of implantation. It progressively resolves during the course of weeks, principally through resorption by macrophages (139). Thrombus resolution and healing in porcine arteries closely reflect the healing in humans after stent implant. Near-total fibrin and thrombus resorption is a feature of complete arterial healing. Proven restenosis therapies such as vascular brachytherapy and DESs impede healing, and treated arteries often show unresolved fibrin thrombus (microscopic or sometimes gross) at times much later than found in untreated arteries.

Inflammation. Thrombus resolves by inflammatory cells (2,140,141). Macrophages secrete a variety of thrombolytic enzymes that digest thrombus as the macrophage tunnels into thrombus surrounding stent strut sites. Inflammation also may occur without thrombus, stimulated by local cytokines. Platelets and their contents appear in thrombus after degranulation and provide major chemokines for inflammation. These include P-selectin and integrins such as beta$_2$ integrin Mac-1 (CD11b/CD18) (142). This integrin, located on the monocyte cell surface, is important because it is prominent in adhesion. Heterotopic platelet aggregation, a process where platelets aggregate on the monocyte surface and stimulate additional platelet activation, also plays an important role. The chemokines also are key for inflammation at vascular injury sites. Monocyte chemoattractant protein-1 attracts monocytes and activated T cells to vessel injury sites.

Inflammation is a potent and direct stimulus for neointimal thickening, in part through stimulating cell proliferation (143). Several animal models exhibit inflammation (monocytes/macrophages, lymphocytes, neutrophils) from stent coatings and drug-releasing polymers. These models suggest that biomedical polymers in DES applications cause inflammation to variable degree in proportion to the polymer mass on the stent. A major challenge in DES technology has been to find polymers that can control drug elution over the course of time yet incite minimal inflammation. Minor inflammation is presently acceptable, as evidenced in guidelines for testing DESs. The "perfect" polymer remains unknown, and all polymers in use today induce some degree of inflammation. It is for this reason that DESs tested in animal models should include quantitative inflammation measurements. A commonly used quantitative assessment of inflammation method is by Kornowski et al. (143).

Cell migration and proliferation. Cell migration and proliferation remain ill-defined in both animal models and in human neointimal hyperplasia. Although cell proliferation is implicated universally in neointimal hyperplasia, its quantitative role remains unclear. Early controversies about the role played by proliferation remain unresolved (144,145).

Both ionizing radiation and drugs effective against restenosis inhibit cell proliferation but have many additional cellular effects, including inhibiting migration, cell signaling, activation, and secretion, and may impair other important reparative features such as angiogenesis (146). These strategies are effective against neointima in multiple animal studies (147,148).

Therapies that are more specifically targeted at proliferation show less clear results. Gene therapy has been used in this strategy, for example, to express cell-cycle inhibitors (p21, p27, p53, and Rb) (149–151) or by halting cell cycle progression by inhibiting CDK2, cdc2, E2F, PCNA, myc, and myb (152–157). These gene-based strategies are marginally successful in animal models and have not been tested in clinical studies. Current DES success using rapamycin and paclitaxel rely on a multitude of cellular targets in addition to proliferation (158,159). The relative contribution of alternative effects is unknown but under investigation.

TIME COURSE OF CORONARY ARTERY HEALING AFTER STENTING

Coronary artery healing after stenting is reported for both the porcine model and in patients. Table 2 summarizes this information. Stent healing in pigs compared with patients suggests a time comparability of approximately 1:6 porcine: human, with pigs healing more rapidly. Reasons for the more rapid process in pigs are unclear but may include the young age of pigs, normal arteries compared with diseased human vessels, and other, as-yet-undetermined factors.

In the porcine model, coronary arteries typically are studied at 1, 3, 6, and 12 months. Although these times are now standard, the reasons for time points after one month principally relate to safety because few changes occur in the pig model beyond this time, with the exception that neointima thins slightly later in the course of time. An unproven concept is that safety requires longer follow-up in pigs (presuming good results at one month) and that this theory might translate to long-term patient safety. The key to a safety evaluation in pigs is complete arterial healing, with thrombus resorption, minimal residual inflammation, and complete or near-complete endothelialization.

Table 2. Time Course Comparison of Events in Porcine and Human Coronary Stenting

	Porcine Coronary Model	Human Stent Implantation
Thrombus	0–14 days	0–30 days
Inflammation	1–14 days	0–30 days
Endothelialization and granulation tissue	4–16 days	14–90 days
Smooth muscle cells and matrix formation	14–28 days	2–6 months

ANGIOGENESIS

Animal models exhibit angiogenesis at arterial lesion locations (Fig. 3). Marked disorganized angiogenesis occurs at stented sites in normal, non-diseased arteries for ill-defined reasons. Vascular hypoxia may be one cause and may result from the compression of adventitial vasa vasorum. Several angiogenic cytokines are upregulated in hypoxia, the most well known being hypoxia-inducible factor-1 alpha. Human atherosclerotic lesions are similarly angiogenic, especially in chronic total occlusions (146,160).

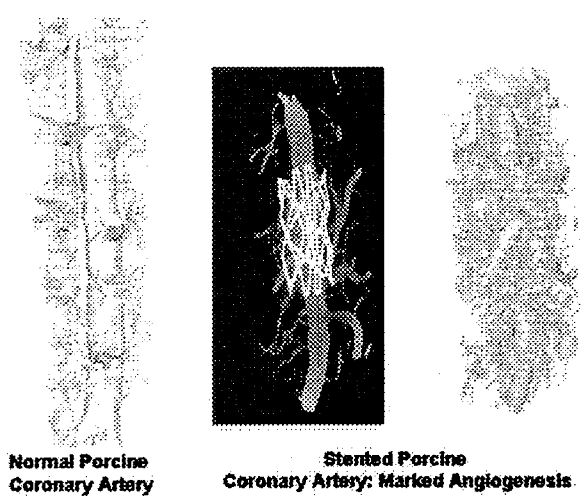

Figure 3. Microscopic computed tomography examination of normal **(left)** and stented **(middle and right)** porcine coronary arteries. Massive angiogenesis results in a highly vascular but disorganized array of vessels after the stenting of a normal porcine coronary artery. (Image courtesy Dr. Hyuck Moon Kwon.)

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LESSONS FROM ANIMAL MODELS: SYSTEMIC RESTENOSIS THERAPIES

Most systemic restenosis treatments have failed and the literature contains many review articles on this topic (161–163). The Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial, which tested oral tranilast to limit restenosis, is the most recent. Several animal studies showed neointimal hyperplasia was reduced in drug-treated animals and suggested oral tranilast efficacy. In one study, rabbits fed cholesterol showed inhibition of neointimal area by tranilast (300 mg/kg) (164). Another study in overstretched porcine coronary arteries showed a 37% reduction in neointimal area normalized to fracture length (147). These and several other preclinical studies preceded the PRESTO trial (147,164–166).

Early small clinical trials showed tranilast could inhibit restenosis, prompting the large, randomized double-blind PRESTO trial of 11,484 patients (167). Primary end points were death, myocardial infarction, and ischemia-driven target vessel revascularization at nine months. Results showed a 15.8% event rate for placebo and 15.5% for tranilast (p = NS). The quantitative coronary angiography substudy comprised 2,018 patients and found that follow-up minimum lumen diameter (MLD) was 1.76 ± 0.77 mm in the placebo group compared with 1.78 ± 0.76 mm (p = NS). Intravascular ultrasonography showed no difference in plaque volume across tranilast doses. Thus, the PRESTO trial was analogous to events 10 years earlier in the Multicenter European Research Trial with Cilazapril after Angioplasty to Prevent Transluminal Coronary Obstruction and Restenosis (MERCATOR) and Multicenter American Research Trial with Cilazapril After Angioplasty to Prevent Coronary Obstruction and Restenosis (MARCATOR) trials (68). Each of these clinical trials was based on early preclinical data that were reported to show efficacy of the drug in question. Subsequent large, randomized clinical trials failed to show any efficacy.

The literature has many reports of preclinical systemic therapies that suggest efficacy of various pharmacologic agents. However, before beginning clinical trials, several important questions must be evaluated. Preclinical studies must use comparable drug doses and obtain comparable drug levels to those planned for clinical trials. Preclinical studies should use the same end points as used in clinical trials, which should include angiographic percent stenosis, absolute lumen MLD, late loss, or intravascular ultrasonography (IVUS)-based, lumen or neointimal parameters (3,145,168,169). An important reason for false-positive preclinical results may arise from histopathologic measurements differing from clinical indices. Such preclinical histopathologic measurements not available or not used in clinical trials include the intima:media ratio, percent neointimal reduction, or microscopic (but statistically significant) neointimal area inhibition. Animal model efficacy reports may yield different conclusions if angiographic or IVUS parameters were standard. The best animal model metric to correlate with clinical data is an area of active investigation.

LESSONS FROM RESTENOSIS MODELS

Safety. Animal models play an instrumental role in developing and improving DES technology, a role that continues to evolve. Safety is the principal concern for any stent technology, and animal models appear useful in its assessment. The critical failure mode for stents is acute, subacute, or late closure because stent thrombosis nearly always has catastrophic implications. The porcine coronary stent model appears predictive for stent thrombosis. Several early studies of brachytherapy in pigs suggested that stent thrombosis might be a problem. Kaluza and Raizner (170) performed balloon and stent injury in healthy porcine coronary arteries, followed by intracoronary beta radiation. Five of 10 pigs given radiation died (50%) of stent thrombosis, whereas none died in the control (non-radiated) group. Stent thrombosis in the porcine coronary model is distinctly unusual, and subsequent patient studies of gamma brachytherapy showed subacute thromboses of up to 14% before the understanding that new stents should not be placed at brachytherapy sites (171–174). The relationship of porcine neointima after brachytherapy to comparable human studies is unclear. Several models show stimulation of neointimal hyperplasia by radiation, whereas clinical studies to date show no evidence of similar problems, at least in the near term.

Neointimal stimulation, rather than its suppression, is a second concern for stent safety, especially with DESs. Toxicity induced by high local drug concentration remains an ongoing concern and can show significant arterial changes. Although rabbit iliac arteries implanted with actinomycin-D showed good results (Fig. 4), the porcine coronary model appears to have predicted enhanced neointima in patients receiving actinomycin-D releasing stents by showing poor healing and neointimal stimulation (Fig. 5). These model studies showed incomplete stent healing, microthrombus, incomplete endothelialization, and late medial necrosis with marked neointimal thickening. The Actinomycin Eluting Stent Improves Outcomes by Reducing Neointimal Hyperplasia (ACTION) trial tested actinomycin-D elution in a randomized study. The trial was halted after 90 of 360 planned patients were enrolled and restenosis rates reached 28% in the highest dose group, suggesting neointimal stimulation (data shown at ESC 2002, Berlin, Germany). High restenosis rates also occurred in lower-dose groups; 25% and 17% for 2.5 μg and 10 μg actinomycin-D, respectively, versus 11% in controls.

Similarly predictive results from animal models were found using very high-dose taxane released from a suboptimal polymer, where the porcine model (Fig. 6) predicted worse clinical restenosis at 12 months. The Study to COmpare REstenosis rate between QueST and QuaDS-QP2 (SCORE) trial was stopped after enrolling 266 of 400

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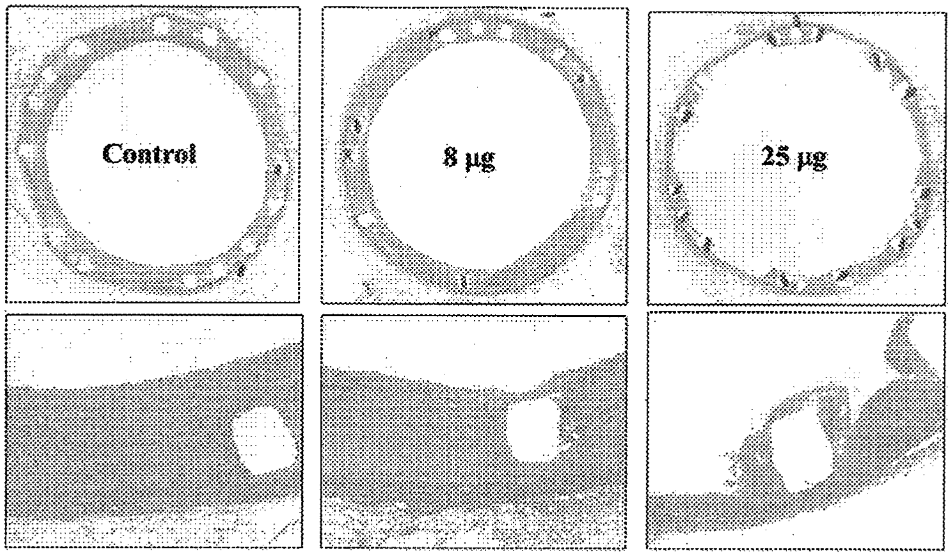

Figure 4. Actinomycin-D studies in rabbit iliac arteries. These images show excellent neointimal inhibition at 8 μg and 25 μg doses **(middle and right columns)**, respectively, compared with control **(left column)**. **Lower rows** are higher power views, showing that the 25-μg dose appears cytotoxic with poor healing present.

planned patients because of late events and increased 12-month restenosis rates (175).

These combined data suggest the porcine model can determine stent safety from both thrombosis and neointimal stimulation perspectives. Increased stent thrombosis in porcine coronary arteries should warn investigators about increased clinical thrombosis risk. Adverse vascular pathologies showing poor healing, vessel toxicity (for example, medial necrosis or cell death), absent endothelialization, or neointimal stimulation should be of major concern.

DES efficacy. The accuracy of efficacy assessment for DESs in preclinical testing remains less clear than their safety. Because restenosis in the stent era is virtually all neointimal thickening, limiting neointima should translate directly from animal models to patients. Unfortunately, this

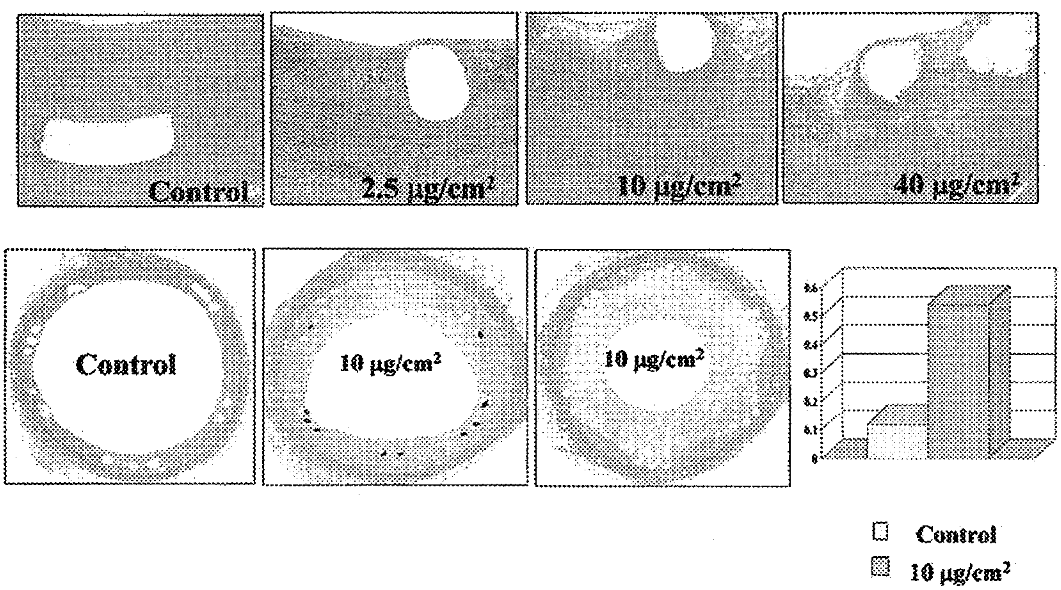

Figure 5. Porcine coronary arteries at 28 days **(top)** and 90 days **(bottom)** after actinomycin-D eluting stent placement. The 28-day data show substantial residual fibrin, inadequate vascular healing, but little mature neointima. At 90 days, there is a marked increase in neointimal thickening, greater than control, which occurred over time. The **graph at lower right** shows neointimal thickness measures for 90-day control and 10-μg datasets. The ACTION trial of actinomycin-D elution was stopped prematurely because of elevated major adverse clinical event rates due in part to abnormally high late loss.

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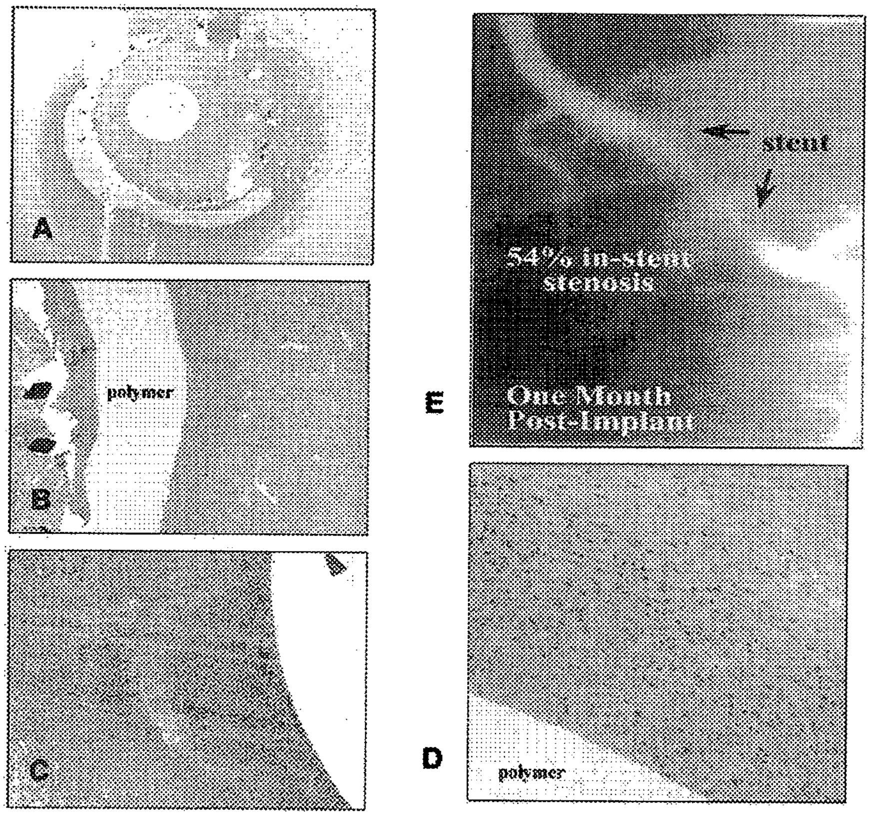

Figure 6. Photomicrographs of porcine coronary arteries at 28 days after the implantation of Quannum-DS (Quanam Medical Corp., Santa Clara, California) stents. These images show neointimal stimulation by high-dose taxane in these stents. Similar results occurred in the SCORE clinical trial. Low power histomorphometry of mid-stent cross-section shows marked vessel lumen narrowing from neointimal hyperplasia. **(B, C, and D)** Rampant inflammation at sites of the polymer-drug combination in these vessels is shown. Several areas of granuloma and hemorrhage **(B)** are present. The inflammation was likely a major cause of the neointimal thickening. **(E)** Cine-film frame of Quannum stent showing marked in-stent restenosis.

translation may not be as direct as desired, and the quantitative relationship between neointima in the porcine model and in patients remains poorly defined.

At least two DESs (rapamycin and paclitaxel) show convincing restenosis efficacy in patients. Both use a compatible polymer for controlled drug release. Suzuki et al. (100) examined rapamycin-eluting stents and compared them with bare stents, dexamethasone-eluting, and both rapamycin-eluting and dexamethasone-eluting devices. The rapamycin-eluting stents reduced in-stent neointimal hyperplasia at 28 days with a mean neointimal area of 2.47 mm^2 (rapamycin alone), 2.42 mm^2 (rapamycin and dexamethasone), 5.06 mm^2 (bare stent), and 4.31 mm^2 (dexamethasone alone). Gallo et al. (150) examined intramuscular rapamycin given to pigs for 14 days after balloon-induced injury. The animals were studied 28 days after percutaneous transluminal coronary angioplasty and showed coronary stenoses of 63% and 36%, respectively (lumen area 1.74 mm^2 vs. 3.3 mm^2; control vs. rapamycin). These two preclinical studies suggest that rapamycin has efficacy against neointimal formation in the porcine artery injury model, a suggestion that was confirmed by clinical trials.

Drachman et al. (176) compared paclitaxel-eluting stents with controls in rabbit iliac arteries after balloon denudation. These investigators found that paclitaxel-eluting stents markedly inhibited neointimal thickening at all late time points and concluded this technology was effective against neointima beyond the time of paclitaxel elution.

Preclinical porcine data used for regulatory submission of the TAXUS stent (Boston Scientific, Natick, Massachusetts) showed the device was safe but also showed no significant efficacy reducing neointima at 28 or 90 days compared with bare metal stents. TAXUS stent clinical data show excellent results at nine months for limiting restenosis (177). Earlier studies of the TAXUS stent are now in their third year and show major adverse clinical event rates of 3% compared with 10% in bare-metal stents. The comparable porcine model data show no change at 180 days from 90 days (unpublished data, personal communications). These crude comparisons suggest that safety, but not efficacy, can be predicted from low-level stent injury (balloon:artery ratio 1.1:1 or less) in the porcine model. Further analysis of paclitaxel animal model data and possibly new models may find application in better predicting clinical efficacy.

SUMMARY

What have we learned from animal restenosis models? Several important principles summarize restenosis models for evaluating DES technologies. These are as follows:

1. Arterial and vascular injuries remain major determinants of neointimal thickening, and mechanical stent designs should limit arterial injury as best as possible.
2. Neointimal formation on DESs develops the same as in bare-metal stents. Thrombus and inflammation play key roles in forming human neointimal hyperplasia, and the polymers used in drug eluting stents incite mild inflammation. Optimal polymer selection may help to minimize this inflammation, and healing within DESs should be documented.
3. Although DESs limit neointimal formation, they may also delay or cause incomplete healing to a greater degree than in bare-metal stents. This is manifested clinically as incomplete endothelialization, unresorbed fibrin deposits, and drug effects typically consisting of hypocellular tissue near the drug-eluting struts. Because neointimal hyperplasia is a normal healing response, some degree of neointima, not obstructive to the lumen, is a desirable outcome for DESs.
4. Animal models, specifically the porcine coronary and rabbit iliac arteries, provide useful information regarding stent thrombosis risk in clinical trials and are thus a measure of safety. All animal studies should carefully determine causes of unexpected preclinical animal deaths, and tabulate stent thrombotic events.
5. Lumen loss in animal models results from several causes. These include medial or arterial cell death, inflammation, and neointimal thickening, results that have correlation in clinical trials. Poor preclinical results mandate strong caution in initiating clinical trials.
6. Efficacy testing in preclinical models has proven difficult to establish. It is unclear whether this is because current animal models do not accurately reflect the human coronary artery response to such stents or whether other causes need be sought. Prior preclinical studies with positive results that did not translate to patients may be due to improper or biased variable selection, or confounding effects of vascular injury.

What must yet be learned from animal restenosis models? The science of preclinical restenosis models is a rapidly developing field and is undergoing intense study. What follows are several key but unanswered questions concerning restenosis models.

Incompletely healed vessels occur in the preclinical DES models. The importance of healing, with incomplete or absent endothelialization, unresorbed fibrin deposits, low-level inflammation, and medial cell dropout is not well understood. For example, the porcine coronary artery safety appears predictive of clinical safety. Actinomycin-D-eluting stents showed nonhealing to a large degree, stimulating porcine neointima. It is uncertain whether improved-yet-incomplete healing will similarly enhance neointimal formation.

The best variables to correlate preclinical models with clinical trials are unknown. Correlative research must be performed to determine which preclinical variables best translate quantitatively to clinical trials. Clarification of whether quantitative measurements of MLD, late loss/loss index, and IVUS-based measurements of neointima in the porcine model will translate well, or if at all, to clinical data. Careful preclinical studies should be conducted for comparison with clinical trials. It is suggested in the interim that angiographic and IVUS end points may best for study in patients, and these combined with histomorphometric data in animal trials should be the best obtainable.

The relative utility of different species is uncertain. Differences between rabbit and pig models must be examined to determine which best translate to patients. This point is key in the prediction of human clinical data from preclinical studies, and we must better understand whether safety (thrombosis and neointimal stimulation) translate for each model to clinical trials.

The optimal time point for termination in animal studies needs clarification to best predict human clinical results. Standard times for animal models are 28, 90, 180, or 365 days, and early positive animal data may become negative at later time points. The time course of arterial healing in animal models bears an uncertain relationship to patients and also must be better understood so that preclinical observations will yield accurate prediction for clinical trials with patient data. Model data at two-year to three-year time points may need examination and correlation with clinical results for accuracy. Additional time points may be important, but presently no clear answer is forthcoming. The time to endothelial recovery for different drug/polymer/stent configurations in injured vessels remains unknown and needs determination.

Several preclinical model enhancements are needed. More rapid turnaround time would be of substantial benefit because current preclinical data can take nine months or longer to process and evaluate. It may be possible that preclinical histomorphometric data (neointimal thickness, histopathologic percent stenosis, lumen size) can be predicted from preclinical quantitative coronary angiography and IVUS in the same animal premortem or postmortem. These parameters might provide a link with human data, and if true would be a major contribution to research and development in drug eluting stents.

Preclinical models are important but imperfect standards, having served the interventional community well for many years. Substantially more remains to be learned, especially regarding the positive predictive results in such models. Active research is aimed at developing a simple, inexpensive, rapid, and accurate preclinical model for human restenosis. This goal is achievable but will require thoughtful direction. Such a model will see rapid adoption for testing, evaluating, and

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prediction and will continue to teach the interventional community important lessons about revascularization therapy.

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Restenosis After Balloon Angioplasty

A Practical Proliferative Model in Porcine Coronary Arteries

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A model of proliferative human restenosis was developed in domestic pigs by using deep injury to the coronary arterial media. Metal wire coils were delivered percutaneously to the coronary arteries of 11 pigs with an oversized, high-pressure (14 atm) balloon and were left in place for times ranging from 28 to 70 days. During placement, the balloon expanded the coils and delivered them securely within the arterial lumen. Light microscopic examination of the vessels confirmed fracture of the internal elastic lamina by the coil. An extensive proliferative response occurred in 10 of the 11 pigs and was associated with a luminal area narrowing of at least 50% in all but one pig. The histopathologic features of the proliferative response were identical to those observed in human cases of restenosis after angioplasty. Immunohistochemical studies confirmed the prominence of smooth muscle cells in the proliferative tissue. A similar response was obtained in two of five porcine coronary arteries in which balloon inflation only was performed, without coil implant. This model is practical and inexpensive and closely mimics the proliferative portion of human restenosis both grossly and microscopically. Thus, it may be useful for understanding human restenosis and for testing therapies aimed at preventing restenosis after balloon angioplasty or other coronary interventional procedures. (*Circulation* 1990;82:2190–2200)

Despite the high initial success rate and widespread use of percutaneous transluminal coronary angioplasty (PTCA), restenosis appreciably limits the effectiveness of this valuable revascularization method.[1-5] Restenosis occurs in 25–45%[6,7] of all patients within 6 months, and attempts to pharmacologically prevent or reduce it using antiplatelet agents,[8,9] anticoagulants,[9] corticosteroids,[10] and calcium channel blockers[11,12] have been unsuccessful. Mixed results have been reported with oral fish oil therapy[13,14] and aggressive lipid reduction.[15,16]

Lack of a practical animal restenosis model has limited the ability to investigate potential therapies. If such a model were available, it might have the additional benefit of yielding insight into the mechanisms of the restenosis process itself. This report describes an experimental animal model of human coronary restenosis developed in domestic swine that accurately mimics the proliferative component of human restenosis and is practical as well as inexpensive.

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Methods

Animals

All studies were carried out with the approval of and with adherence to the guidelines of the Mayo Clinic animal care committee.

The coronary arteries of domestic crossbred pigs (*Sus scrofa*) are comparable with those of humans in morphology and microscopic structure. This animal was thus chosen as one in which the coronary vessels might respond to injury similarly to the coronary vessels of humans. The carotid arteries of this animal have been studied previously[17] as a model for the effects of balloon dilation.

Juvenile pigs (20–30 kg) were obtained from local farmers and fed a standard laboratory chow diet without lipid or cholesterol supplementation throughout the study.

Coil Configuration

The coil configuration that was used to produce vessel injury in this model was as follows. A length of wire (0.005-in. tantalum or stainless steel) was formed into a to-and-fro pattern so as to remain in a single plane. This structure was then wrapped about the surface of a cylinder-forming mandril either longitudinally or in a serially helical pattern. The diameter of the mandril was comparable with that of an expanded PTCA balloon (3.0 mm). The coil

structure was then gradually compressed into smaller and smaller diameters and finally crimped on a fully deflated balloon (roughly 1.4 mm in diameter). The resulting three-dimensional configuration causes multiple wires to be present in a given section perpendicular to the vessel long axis.

Inflation of the balloon resulted in expansion of the coil to full balloon diameter. This configuration and expansion mechanism are similar to several balloon-expandable intracoronary stent designs, although use of the device to produce the model requires intentional arterial damage inflicted on the vessel wall through gross oversizing.

Procedure

All pigs underwent intramuscular injection of 12 mg/kg ketamine and 8 mg/kg xylazine for anesthesia. They were placed supine, and the ventral neck region was infiltrated with 1% xylocaine (total dose, 10 ml) for local anesthesia. Continuous electrocardiographic monitoring was performed. The right external carotid artery was exposed, and an 8F arterial sheath was placed. Heparin (5,000 units) was administered intravenously as a bolus.

The PTCA balloons (3.0 mm) and metallic wire wrap were inflated such that the balloon would deposit the coil securely in place within a coronary artery. The balloon size (3.0 mm) was substantially larger than these pig coronary arteries, which are typically 1.5–2.5 mm in diameter.

The left main or right coronary artery was engaged using standard techniques with an 8F PTCA guide catheter under fluoroscopic visualization. To engage the left main coronary artery from either carotid artery, a standard right Judkins JR4 curve was used. Conversely, to engage the right coronary artery, a standard left JL4 curve was used. Thus, the left/right engagement methods are reversed from those used in the human femoral artery approach. The balloon/metallic coil device was advanced into either the left anterior descending, circumflex, or right coronary artery over a 0.014-in. PTCA guide wire. The balloon was inflated once to high pressure (14 atm), deflated, and removed. Another bolus of heparin (5,000 units) was then administered. Fluoroscopy and selective contrast injection confirmed both vessel patency and coil location. Repeat angiography was performed within 15 minutes to confirm vessel patency. The carotid vessel was repaired, using standard techniques, or ligated, and the neck wound was closed with interrupted sutures. The pigs were returned to quarters and closely observed. No antiplatelet agent was used at any time, and no additional heparin was given.

To determine the response of the coronary vessels to oversized, hyperbaric balloon inflation only (without coil implant), the procedure was performed identically except that a PTCA balloon was used without a metallic coil mounted on it. This latter procedure was performed in five pigs. An additional three pigs underwent coil implantation in which the coil was matched more closely to the vessel diameter, in an effort to establish the fact that oversizing the coil is an essential part in the production of medial injury and vessel response.

Histopathology

Pigs were killed at times from 28 to 70 days by using intravenous barbiturate and KCl. Two pigs died spontaneously at 9 and 11 days after coil implant. All hearts were removed immediately after death and perfusion-fixed at 100 mm Hg for 24 hours with 10% neutral buffered formalin. Those coronary artery segments containing the metal coils were easily identified externally.

These segments were carefully removed from the heart with at least 1 cm of normal vessel proximal and distal to the coil. Gross sectioning of the fixed vessels was performed at 2-mm increments perpendicular to the vessel axis. Coils were left in place, and cutting was done with sharp, hardened scissors. Individual coil wires were cut first, followed by the arterial tissue. This method resulted in minimal vessel size and shape distortion before embedding in standard paraffin block.

Each embedded arterial segment was cut and stained with hematoxylin-eosin and Lawson's elastic–van Gieson stains. Immunohistochemical stains including actin, desmin, and vimentin were performed on a subset of three pigs.

Each 2-mm histological section was examined to determine the site of maximal luminal narrowing for a given artery. The section with the most severe stenosis was used to measure the following parameters: major and minor axes of the native vessel lumen (measured from internal elastic lamina to internal elastic lamina across the largest and smallest diameters) and major and minor axes of the stenotic lumen (residual lumen diameters). Percent area stenosis was calculated assuming the lumen to be an ellipse (area$=\pi\times$major axis$\div 2\times$minor axis$\div 2$). Measurements were made microscopically using a calibrated eyepiece reticle.

All sections were examined by an experienced cardiac pathologist (W.D.E.) for comparison with human restenosis tissue in regard to cell type, architecture, and amount of ground substance. The human tissue for comparison was obtained previously from patients undergoing directional coronary atherectomy for the treatment of restenosis.

Results

Coil Implantation

Eleven pigs underwent successful coil implantation and survived chronically. During this same time period of successful implants, coil implantation attempts were made in an additional eight pigs, all of which died acutely (within 6 hours of implantation) for the following reasons: there were four anesthetic and procedural deaths and four deaths related to

TABLE 1. Survival and Coil Characteristics in Coil-Implanted Pigs

Animal number	Days survived	Coil material	Coil location
1	67	Tantalum	RCA
2	53	Stainless	LAD
3	69	Tantalum	RCA
4	70	Stainless	LAD
5	69	Stainless	LAD
6	11*	Stainless	LAD
7	57	Tantalum	CX
8	28	Tantalum	CX
9	28	Stainless	LAD
10	28	Tantalum	CX
11	9*	Tantalum	LAD

RCA, right coronary artery; Stainless, stainless steel; LAD, left anterior descending coronary artery; CX, circumflex coronary artery.

*Spontaneous death; remaining animals were euthanized.

severe coronary artery injury by the coil itself. Overall survival was thus 11 of 19, or 58%.

All pigs had patent vessels, determined angiographically within 15 minutes of coil implantation. Two pigs died at 9 and 11 days, respectively, after coil implantation. At autopsy both of these pigs showed extensive proliferative neointimal tissue with severe stenosis of the vessel lumen. No acute thrombus was observed in either pig at the site of the coil-induced stenosis. Thus, it was assumed that these severe stenoses rendered each heart ischemic during normal activity and caused a fatal arrhythmia. In the pig heart, vulnerability to ischemic ventricular fibrillation is well known and presumably relates to a lack of collateral circulation.

The remaining nine pigs survived without complications or clinically apparent problems until death by euthanasia (Table 1). Light microscopy in all pigs revealed a proliferative neointimal response of varying magnitude. Figure 1 demonstrates gross stenosis caused by the proliferative neointima.

In all pigs, rupture of the internal elastic lamina by at least some of the metallic coil wires was evident, and the coil usually resided in the vessel media. Figure 2 shows a low-power photomicrograph of another stenotic segment. Rupture of the internal elastic lamina is evident, and the coil wires have been driven entirely through the vessel media. A thick neointima is present, causing significant luminal stenosis. Mild chronic inflammation was usually evident around each coil wire. No qualitative histopathologic differences were noted between the tantalum-implanted versus the stainless steel–implanted vessels.

A normal vessel just proximal to coil placement is shown for reference in Figure 3. Figure 4 is of particular interest because not all wires ruptured the internal elastic lamina. The greatest degree of proliferation resulted from the two coil wires that ruptured the internal elastic lamina, with neointima growing to confluence between them in the vessel lumen. On the contralateral side of the vessel, however, the lamina remained intact, media was not entered, and substantially less smooth muscle cell proliferation is seen. At the bottom portion of this section, normal media without any proliferation is seen. This is the segment with the greatest distance between coil wires.

Table 2 shows the stenotic and native lumen sizes and the resulting percent area stenosis. When examined under higher power, the histological characteristics of this proliferation are identical to those of tissue obtained from 38 humans who had angiographic restenosis after PTCA and underwent directional atherectomy with the Simpson atherectomy catheter. Figure 5 is a side-by-side high-power microscopic comparison of the pig proliferative tissue and a representative sample of human restenosis tissue. That these proliferative tissues (human and porcine) are virtually identical is evident in terms of cellular appearance, cell density, and amount of intercellular ground substance. Immunohistochemical stains (actin, desmin, and vimentin) in the porcine tissue showed that these proliferative

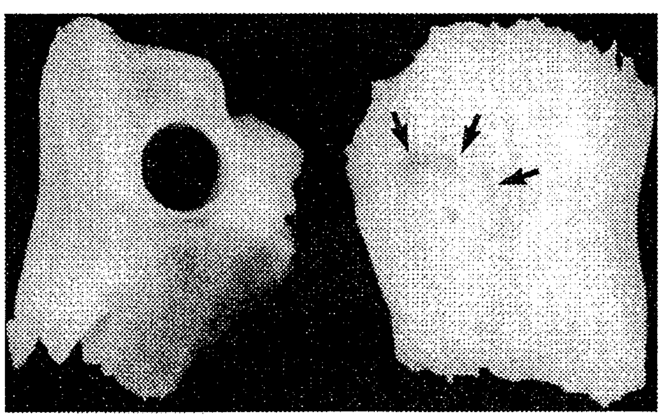

FIGURE 1. *Gross photograph of luminal compromise resulting from the metallic coil placement. These cut sections were taken from the same left anterior descending coronary artery, within 3 mm of each other. The implantation of coil wires is shown in the proliferative section (arrows, right), while a normal appearing vessel is seen where there were no coil wires (left). The proliferation induced by the injury nearly obliterated the lumen of this vessel, resulting in a severe stenosis.*

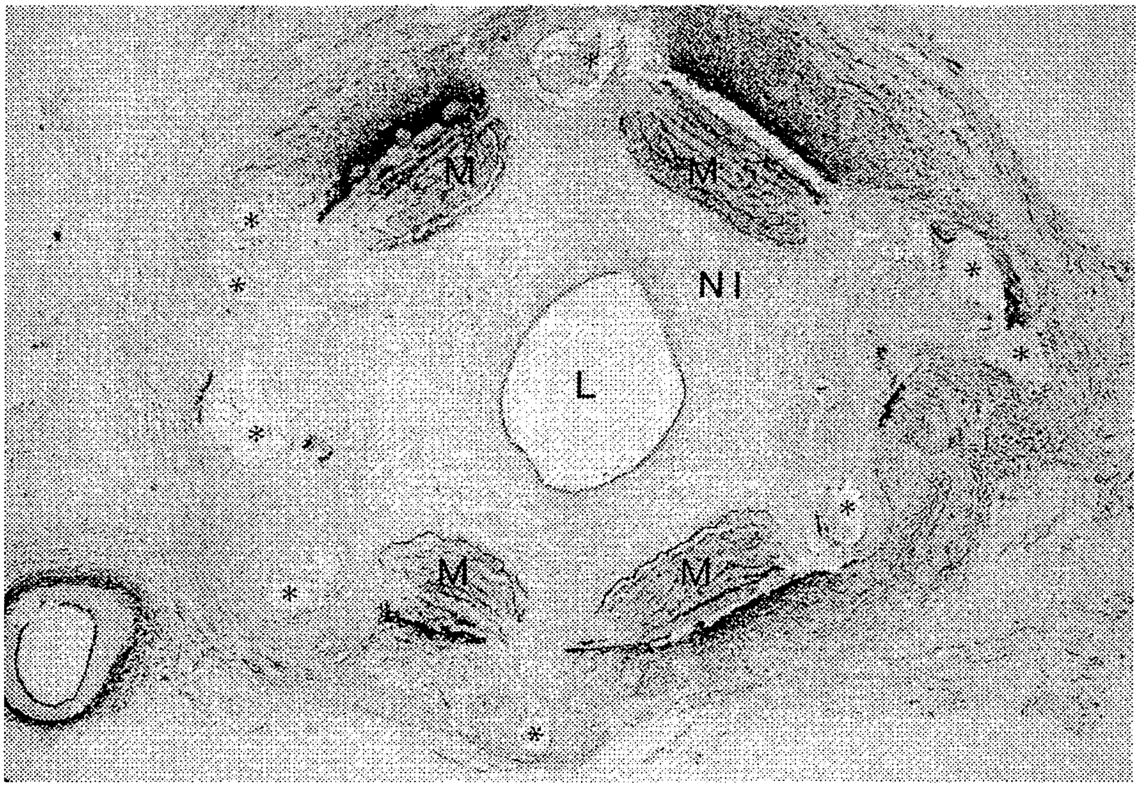

FIGURE 2. *Photomicrographic section shows gross neointimal proliferation causing a significant stenosis. Elastic–van Gieson stain was used. The gross proliferation and luminal compromise by neointima is obvious. Also, destruction of the internal elastic lamina by the coil wires is easily seen. L, lumen; NI, neointima; M, media; *, holes from coil wires. Magnification, ×30.*

cells were of smooth muscle origin, evidenced by the strong presence of actin and vimentin and significantly less desmin.

Balloon Inflation Only

Five additional pigs underwent oversized, overpressured balloon inflation only, without coil implantation. Table 3 shows results from this series. Three of these five pigs had a proliferative response to deep medial injury although the percent stenosis was somewhat less in two. In one pig, there was complete occlusion, but this was from acute thrombosis and, in retrospect, represented excessively severe oversizing of the balloon to the vessel (a small diagonal artery). The remaining two pigs had little or no proliferation seen. Figure 6 depicts one of the two vessels that underwent proliferation and moderate luminal obstruction.

Coil Implantation, Not Oversized

The three pigs with coil implantation in which coil size was closely matched to vessel size did not exhibit appreciable proliferation. Figure 7 shows the minimal amount of neointimal proliferation in a representative animal from this group.

Discussion

Efforts to reduce or eliminate restenosis after PTCA have largely been unsuccessful. These efforts have been hampered by a lack of knowledge regarding the pathophysiological mechanisms of human restenosis and the lack of an accurate animal restenosis model with substantial proliferation. Histopathologic observation of restenotic tissue from living patients has become readily available with the advent of directional atherectomy.[18] Given this information, there is considerable interest in identification of an animal model similar to human restenosis.

Other Animal Models

Previous angioplasty animal models have not addressed the proliferative aspects of restenosis directly, concentrating instead on the atheromatous nature of the lesions.[19,20] The model described by Sanborn et al[21] has been frequently used. In this model, rabbits fed atherogenic diets have serum cholesterol levels frequently exceeding 1,000 mg%. The resulting atheromatous lesions of the aorta, iliac, and femoral vessels contain many foam cells in addition to intimal thickening. Although balloon denudation of endothelium increases proliferation,

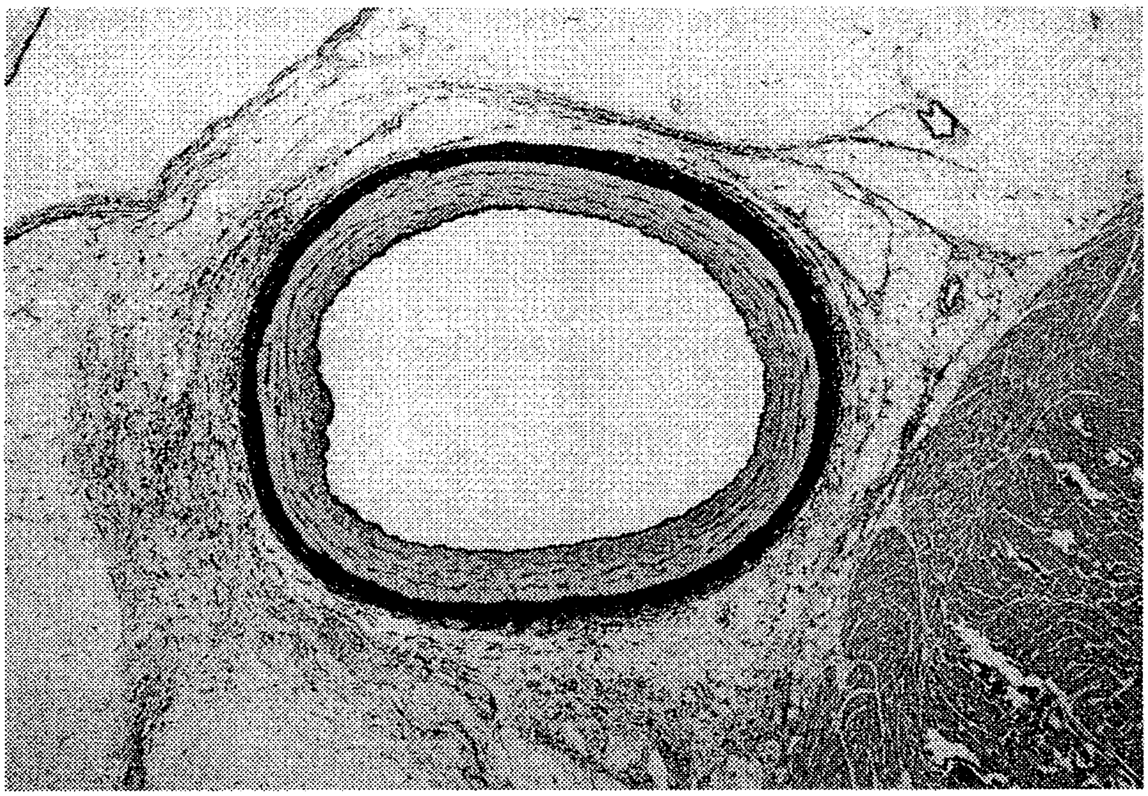

FIGURE 3. *Normal section of coronary artery taken adjacent to a segment with coil placement. The internal elastic lamina remains intact, and no significant proliferation is seen. Elastic–van Gieson stain was used. Magnification, ×30.*

many foam cells are present in contrast to those found in human restenosis. Another model of restenosis in pig carotid arteries involves endothelial denudation with neointimal proliferation. In this model, however, significant proliferative stenoses are not produced unless caused by an occluding, organized thrombus.[17] The carotid or iliac arteries of these models are elastic vessels, as opposed to the coronary arteries (muscular arteries), which contain proportionally more smooth muscle. These noncoronary vessels may thus be less suitable for a coronary artery restenosis model since smooth muscle proliferation is likely a major factor in the genesis of restenosis. The current model results in obstructive lesions histopathologically identical to the proliferative component of human restenosis, in contrast to prior models.

Medial Injury and Restenosis

This model mimics the injury induced by PTCA by causing extensive deep medial injury. Oversizing the balloon for the target vessel results in severe elevations of vessel wall tension. This is followed by chronic tension in the medial smooth muscle due to the presence of the wire coil. Some degree of foreign body irritation also likely results from the wire coil itself. The small diameter wire on the surface of the balloon results in extreme shear stresses from the small radius of curvature of the wire. Many wires thus penetrate the internal elastic lamina into media rather than simply circumferentially distending the vessel.

Figure 4 strongly suggests that extensive smooth muscle proliferation is a response to rupture of the internal elastic lamina and consequent medial injury. Rupture of the internal elastic lamina during PTCA, medial laceration, and subsequent restenosis have been documented[22–24] in humans. Mechanical medial injury is a known factor in generating a proliferative response.[25] It is evident that simple overdistension of the vessel wall alone, without medial injury, does not produce the intense proliferation in this model, since portions of the vessel media that were stretched but not penetrated by wire exhibit mild or no proliferation whatsoever. This model suggests that lacerations or splits of normal media may contribute substantially to the genesis of restenosis.

That a proliferative response was elicited by inflating an oversized balloon suggests that the method of medial injury may not be as important as the injury itself. In the pigs that underwent balloon inflation only, the proliferation was produced less reliably. This reliability factor might be improved with further study, but at present the coil injury method appears preferable.

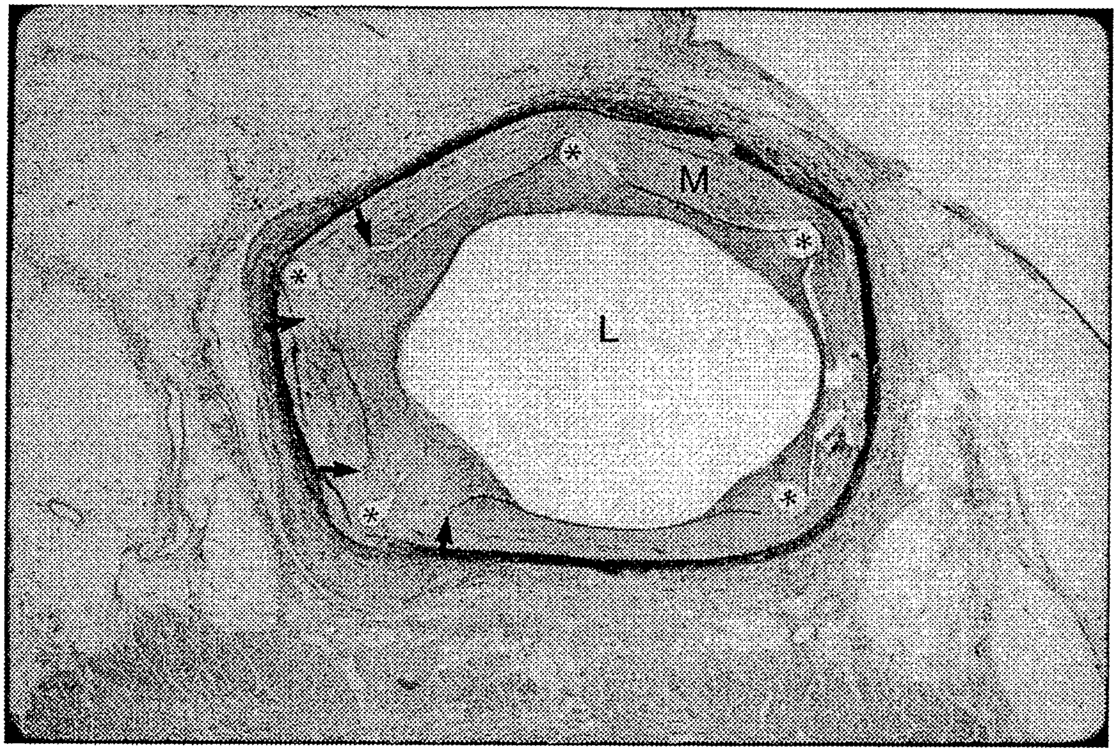

FIGURE 4. *Microscopic section (low power) in a case in which, fortuitously, not all coil wires penetrated into the vessel media. In this section, the two coils farthest left penetrated the media (arrows) and resulted in substantial proliferation. Conversely, the top and farthest right two wires did not penetrate the media, and less proliferation resulted. A short segment of vessel media at the lowermost portion of the figure is entirely normal, without any proliferation, although this segment was stretched by the balloon. This normal appearing segment has the farthest distance between any coil wires. Elastic–van Gieson stain was used. L, lumen; M, media; *, holes from coil wires. Magnification, ×30.*

Closely matching the coil/balloon size to vessel size resulted in minimal proliferation, consistent with the concept that proliferation is proportional to degree of injury. Furthermore, it suggests that the vessel injury resulting from the coil rather than the coil itself is responsible for the proliferation. This obser-

TABLE 2. Luminal Compromise Data in Coil-Implanted Pigs

		Native lumen			Stenotic lumen		
		Diameter (mm)			Diameter (mm)		
Animal number	Area stenosis (%)	Major	Minor	Area (mm^2)	Major	Minor	Area (mm^2)
1	75	1.74	1.71	2.34	0.99	0.75	0.58
2	70	2.94	2.85	6.58	1.65	1.53	1.98
3	18	2.19	1.62	2.78	1.98	1.47	2.29
4	86	2.34	1.38	2.54	0.87	0.51	0.35
5	50	2.70	2.52	5.35	2.43	1.41	2.69
6	72	1.35	1.08	1.15	0.75	0.54	0.32
7	94	1.89	1.44	2.09	0.45	0.36	0.13
8	50	1.95	1.74	2.67	1.32	1.29	1.34
9	99	3.36	2.67	7.05	0.09	0.06	0.005
10	99	2.46	2.01	3.88	0.30	0.18	0.04
11	95	2.40	2.13	4.01	1.41	0.84	0.21

Percent area stenosis=100×[1.00−(stenotic area÷native vessel area)]=100×{1.00−[(π×stenotic major axis×stenotic minor axis÷4)÷(π×native major axis×native minor axis÷4)]}.
Vessel area=π×major axis÷2×minor axis÷2.

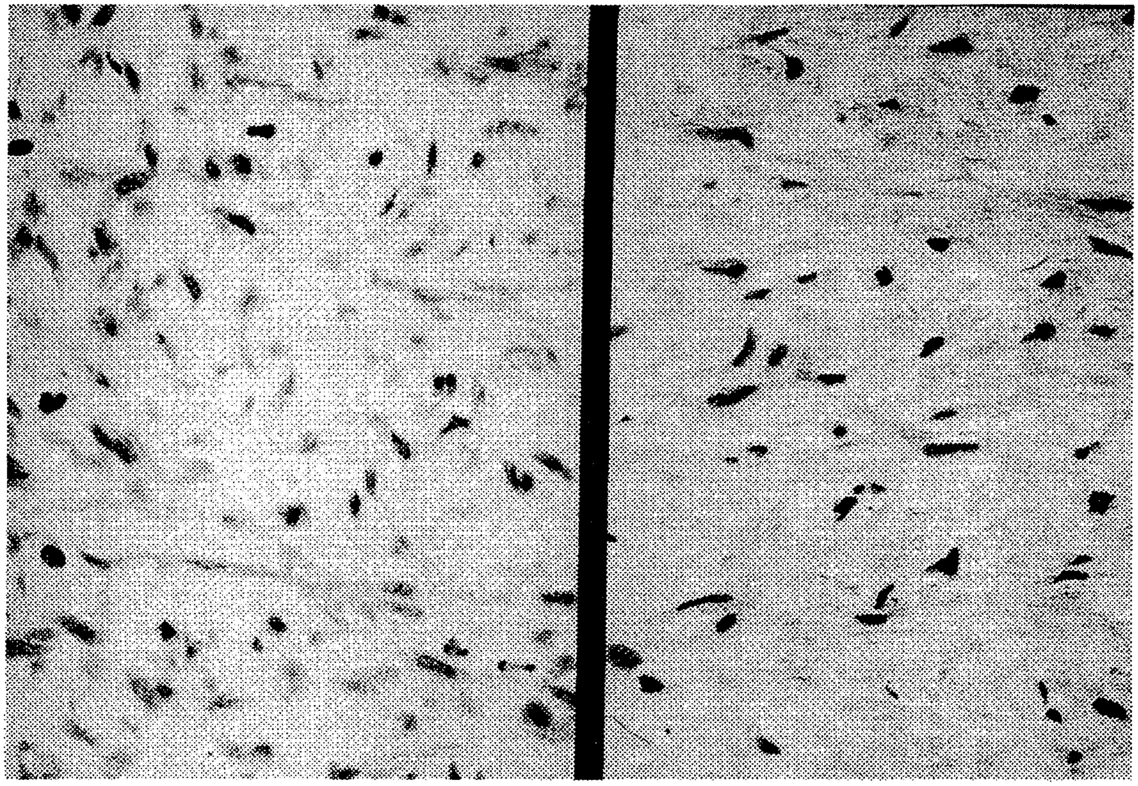

FIGURE 5. *High power side-by-side comparison of a representative sample of human restenosis (left panel) and tissue from the porcine restenosis model (right panel). The character of cells and proportion of ground substance is identical. Hematoxylin-eosin stain was used. Magnification, ×300.*

vation implies that the invasive cardiologist should strive to minimize vessel injury when performing PTCA or other interventional procedures.

Role of Lipids in Restenosis

In contrast to primary atheromatous lesions, the human restenotic lesion consists of a vigorous proliferation of smooth muscle cells that have likely migrated from damaged media into the lumen as part of the reparative process. The proliferative nature of the restenotic lesion thus differs distinctly from the original atherosclerotic disease. The time course of restenosis is appreciably shorter,[26] also suggesting a different mechanism.

No atherogenic diet was fed to the pigs in the present study. The production of histology resembling proliferative restenotic morphology without hyperlipidemia also supports the concept that restenosis is a process independent from atherosclerosis. Hyperlipidemia might intensify the observed proliferative response, a possibility not tested in this study. Although the proliferative effects might have been promoted further with a high cholesterol diet, hyperlipidemia is clearly not a necessary condition for production of the proliferative response in this model.

Foreign Body Response

Stainless steel and tantalum are relatively biologically inert materials. However, both materials stimulated restenoticlike neointima in this model. This may be from the chronic, severe mechanical tension placed on the vessel due to the oversized coil expansion, from a foreign body reaction, or from both. Since only a minimal amount of chronic inflammation was observed in this model, it is likely that inflammation was a lesser factor in stimulating proliferation. This is consistent with the fact that a proliferative response was also produced in pigs that underwent balloon inflation only, with no coil present. That there were no apparent histopathologic differences between the tantalum and stainless-steel coils supports the concept that injury from the coils, and not the coil material itself, caused the proliferation.

Platelets and Thrombus

The role of platelets and thrombus is not well defined in the current model. In the hyperlipidemic rabbit iliac artery, a statistically significant reduction in restenosis was found when antiplatelet agents were used after balloon dilation of a stenotic segment.[27] Platelet deposition and release of growth

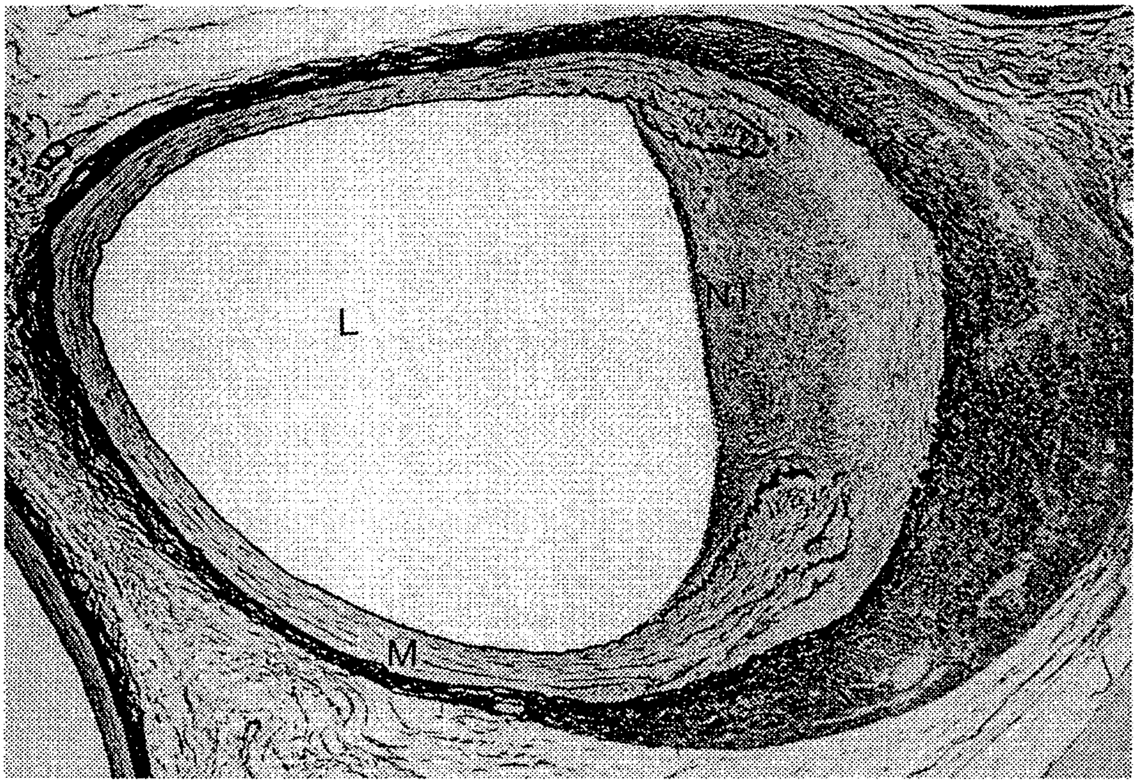

FIGURE 6. *Representative section from one pig that underwent inflation only, without coil implant. Note the proliferative neointima, not as obstructive as in those vessels injured by the coil method. Elastic–van Gieson stain was used. L, lumen; NI, neointima; M, media. Magnification, ×30.*

factors may play a role in the genesis of this model; it was for this reason that no antiplatelet agents were used at any time in this study. Platelet deposition and thrombus at the site of medial injury and on the coil itself would be expected in this model. This deposition could be a factor responsible for the proliferation of smooth muscle cells.[28,29] Prior reports[17] suggest that endothelial regrowth protects against platelet-thrombus deposition. Therefore, it is possible that the initial days after angioplasty when endothelium and neointima are forming may be critical in the genesis of the proliferative response. Aspirin pretreatment of these pigs before and after coil implant might have diminished the proliferative response.

In the current model, the foreign body coil may have slowed endothelial regrowth. Thus, there might have been longer exposure of media to blood elements that increased the amount of platelet deposition, thrombus, and consequent cellular proliferation. Acute studies in this model should be examined to establish the degree of thrombus and platelet deposition at the site of vessel injury.

TABLE 3. Luminal Compromise Data in Pigs That Underwent Balloon Inflation Only

		Native lumen			Stenotic lumen		
		Diameter (mm)			Diameter (mm)		
Animal number	Area stenosis (%)	Major	Minor	Area (mm^2)	Major	Minor	Area (mm^2)
1	54	2.04	1.89	3.03	2.04	0.87	1.39
2	29	3.09	1.68	4.08	3.09	1.20	2.91
3	100*	3.15	1.29	3.19	0.00	0.00	0.00
4	0	3.21	2.28	5.74	...	...	...
5	0	2.94	2.19	5.05	...	...	...

Percent area stenosis = 100×[1.00−(stenotic area÷native vessel area)] = 100×{1.00−[(π×stenotic major axis×stenotic minor axis÷4)÷(π×native major axis×native minor axis÷4)]}.

Vessel area = π×major axis÷2×minor axis÷2.

*Thrombotic occlusion.

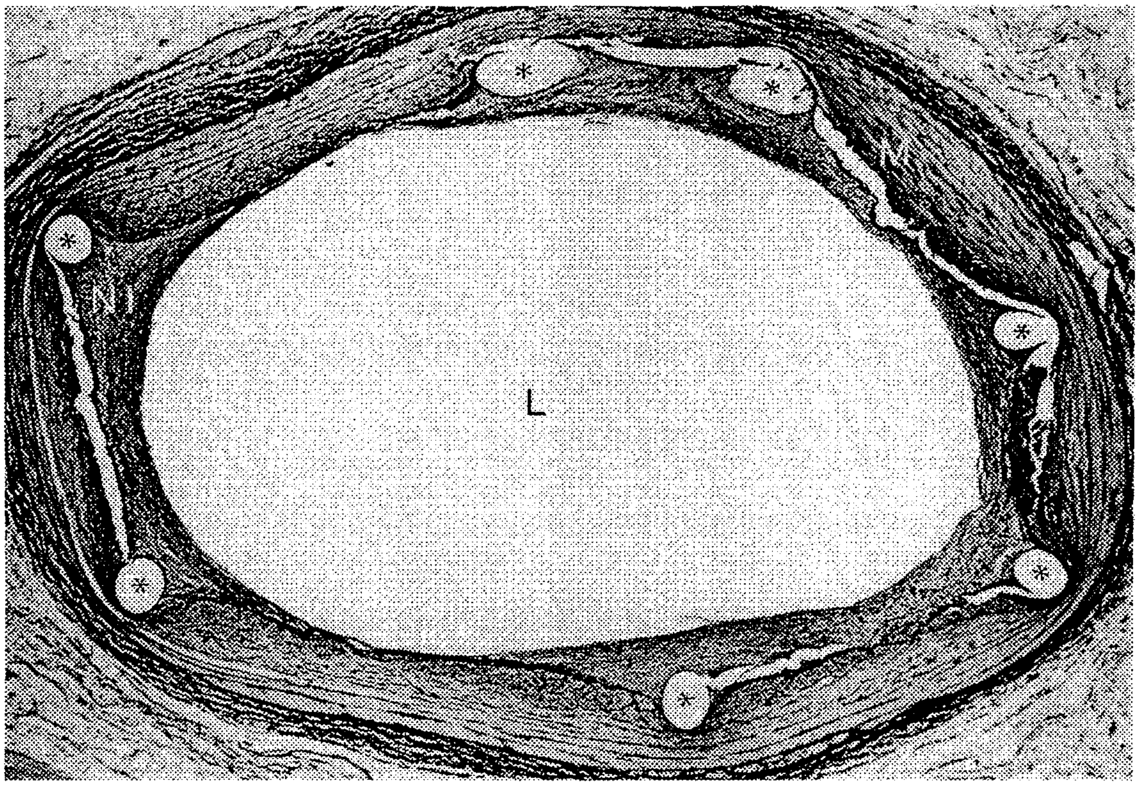

FIGURE 7. *Section from one pig in which the coil was sized more appropriately to the vessel lumen (right coronary artery). The proliferative neointima is comparatively thin and not as obstructive as in those vessels severely injured by the coil method. Elastic–van Gieson stain was used. L, lumen; M, media; NI, neointima; *, holes from wire coils. Magnification, ×30.*

Implications for Coronary Artery Stents

The analogy between the metallic coil implanted in this model to generate restenosis and the current generation of self-expanding[30] or balloon-expandable metallic stents intended to prevent restenosis is obvious.[31,32] The proliferative response in this pig model resulted from intentional, severe oversizing and overinflating the balloon on which the coil was mounted. The intent in this model was to injure media to stimulate a vigorous healing response. Neointimal tissue covering stents in experimental animal stent placement is likely a mild foreign body response but has never been shown to proliferate as severely as noted in this model. Experimental stents have been placed in normal animal vessels, quite different from freshly dilated atherosclerotic human vessels. Restenosis data from human studies with stents are inconclusive, although restenosis despite stenting has been documented with varying incidence.

Nevertheless, there may be important clinical lessons from the induction of substantial proliferation with this coil-injury model. Vessels to be stented should be dilated first by a balloon alone, rather than by using the stent/balloon combination as a primary dilation device. This should be done to minimize damage to the vessel from the extreme shear forces generated at stent wire sites that occur with the stent/balloon catheter combination. If perforation of the internal elastic lamina is indeed responsible for increasing the proliferative response, predilation using a balloon alone should help eliminate further damage to the lamina at stent wire sites.

Although there has been recent speculation that different stent designs might result in lower restenosis rates, this principle has never been scientifically tested either in animal models or in human clinical trials. The current study did not address this principle. The coil configuration was flexible, and a wire size was used similar to that in clinically implanted stents. It is safe to assume that vessel damage from deep medial injury after rupture of the internal elastic lamina resulted in the majority of proliferations in this model since proliferation from nonpenetrating wires was not as severe as when media was injured. Thus, sizing and deployment may be as important as specific stent design and configuration. Designs that are stiff, significantly altering the three-dimensional vessel course, might result in chronic forces that could result in increased vessel damage.

These considerations are appreciably altered in the case of stent placement in an atherosclerotic lesion that has undergone dilation. The primary

reason for stenting is optimizing and maintaining vessel lumen, in opposition to smooth muscle proliferation induced by the stent. These are opposing forces for which an optimum balance must be sought. Extremes on either end may result in less favorable luminal results. It is possible that this model could be used to study some of these factors, especially with regard to optimal stent sizing. Different coil designs might also be tested for relative efficacy at maintaining lumen as a tradeoff against smooth muscle cell stretch and damage.

Conclusion

This porcine model for the proliferative component of human restenosis is accurate and simple and develops in a short period of time. Whereas the model may differ from human restenosis in its mechanism of production, the gross and histopathologic results appear identical to those found in human restenosis. Therapies aimed at reducing the occurrence of restenosis might thus be easily evaluated using this model.

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